

CIGUATERA IN THE FRENCH WEST INDIES

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Ciguatera fish poisoning was investigated on the Island of Saint-Barthelemy, Leeward Islands, Caribbean Sea, from 1979 to 1989. Clinical features include gastrointestinal and neurological disorders. 440 fish caught in fish-pots or by hook and line were checked by mouse and chicken bioassays. Jacks (*Caranx* spp.) and barracudas were highly ciguatoxic. Weight and toxicity were not correlated except for the most toxic species *Caranx laevis*. Small carnivorous fishes classified as invertebrate feeders are likely involved in the transfer of ciguatoxin in the food chain since they contained significant levels of toxin. Herbivores (e.g. surgeonfishes or parrotfishes) which are not locally implicated in ciguatera, contained sometimes low levels of ciguatoxin. *Gambierdiscus toxicus* occurred in coastal waters of Saint Barthelemy but this species may not directly produce ciguatoxin.

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In the Caribbean, ciguatera poisoning is known from the Bahamas (Pestry, 1975) to Martinique (Menger, 1979) including Florida (Lawrence et al., 1980) Cuba (Bagnis, 1979a) and Puerto Rico (Gilman, 1942; Payne & Payne, 1977) with a higher incidence in small Islands such as the Saintes, the Virgin Islands (Czernichow et al., 1984; Hanno, 1981; Morris et al., 1982) and the Leeward Islands (Morice, 1965). One of the Leeward Islands, Saint Barthelemy, is a small (c. 25 km), arid (without river or spring), tropical Island at 17°55'N, 62°50'W (Fig. 1). The 3 adjacent islands of Saint Barthelemy, Saint Martin and Anguilla are surrounded by a wide shelf (max. depth 60 m), with fringing reefs which continue across the shelf. These submerged reefs often form scattered coral-shoals 10–20 m underwater. At the edge of the open sea the shelf falls abruptly to >200 m. This coral reef ecosystem shelters a great variety of reef fishes (Vernoux et al., 1988).

Laboratory research on ciguatera was initiated in this area in the 1980's. It was shown that in fish the poison is lipid-soluble and quite similar to ciguatoxin isolated in the Pacific (Vernoux et al., 1982; Hoffman et al., 1983). Heterogeneity of ciguatoxins extracted from viscera or flesh of Caribbean fish was also demonstrated (Vernoux & Abbad el Andaloussi, 1986) and confirmed (Vernoux & Tahla, 1989; Gamboa et al., 1990). *Gambierdiscus toxicus* (Bergman & Alam, 1981; Besada et al., 1982) was suspected as the ciguatoxin elaborator and as the producer of maitotoxin (Miller et al., 1984). A study of ciguatera poisoning and occurrence of ciguatoxins in fish was carried out on Saint Barthelemy. Results are

presented here, together with results from experiments with *G. toxicus* sampled at Tahiti in 1976 (in Dr. Bagnis's laboratory).

MATERIAL AND METHODS

Fish collected at numerous locations on Saint Barthelemy between 1979 and 1989 were identified from Stokes (1980). Most were caught in fish traps on the sea bottom (depth: 30–50 m) within the reef habitat. King mackerel (*Scomberomorus cavalla*) and ccro (*Scomberomorus regalis*) were collected by trolling, and greater amberjack (*Seriola dumerili*), black jack (*Caranx lugubris*) and african pompano (*Alectis crinitus*) by hook and line. Whole animals or separated tissues were frozen at –20°C for transport to the laboratory and stored until processed.

Lipid-soluble residues (LR) were prepared from flesh or viscera by a routine acetone or methanol method, respectively (Vernoux et al., 1985a). For the qualitative testing, fish liver or LR were fed to chicks as reported elsewhere (Vernoux et al., 1985b; Vernoux & Lahlou, 1986). For quantitative testing a mouse bioassay was used (Vernoux et al., 1985a). The toxin concentration was expressed in Mouse Units gram per gram of tissue (MUg/g), where 1 MUg is the weight-specific minimum lethal dose (1 MUg = 1 MU/20). Fish was assumed to be ciguatoxic when typical symptoms of ciguatera (Vernoux, this memoir) were observed in both chick and mouse.

For experiments in Tahiti in 1975 and 1976, a mixture of algae and detritus was scraped from the surface of dead corals collected at the Gam-



FIG.1. The Caribbean region.

bier Islands. This mixture was fractionated by hand according to size and, then by successive passes through sieves of different mesh sizes (Blutex Nylon: 85µm, 36µm and 10µm). Vortex shaking helped to separate associated organisms. Our routine acetone method was used to extract ciguatoxin in each biodebris fraction. Remaining dry organic matter was treated with boiling methanol (Yasumoto & Endo, 1973) to extract the maitotoxin. This allowed a separation of ciguatoxin (CTX) and maitotoxin (MTX) with little reciprocal contamination, since maitotoxin is poorly soluble in acetone (Yasumoto et al., 1976). These toxins were identified by symptomatology induced in mice and by their chromatographic behaviour on a silicic acid column. For analysing toxins in the gut contents of herbivorous fish the method of Yasumoto et al. (1977a) was used.

Analysis of variance (ANOVA) was done using SPSS-PC software. A non-parametric ANOVA K-W test was also used in parallel.

RESULTS

EPIDEMIOLOGY

With the help of some local physicians, fish poisoning was surveyed at Saint Barthelemy (3000 inhabitants). 10–30 cases were identified per year either from consultations or from patients treated at the hospital. Generally they were fishermen or tourists having consumed small jacks, big mackerels, snappers or seabass.

In some cases, poisoning was induced after a second meal of the same fish. Patients complained of sensitivity disturbances (painful tingling about the mouth and throat, hot and cold reversal) nausea and vomiting, abdominal pain, diarrhoea, myalgia, weakness and hypotension. Visual disturbances (blurred vision) and persistent itching were also noted. After symptomatic therapy, complete recovery occurred after a few days, weeks or months. After a primary poisoning some individuals develop a fish feeding allergy and cannot eat any fresh or canned fish. Toxin analysis of fishes implicated in these ciguatera outbreaks revealed ciguatoxins at >1MUg/g of flesh.

DISTRIBUTION OF TOXICITY

During the course of this study we observed that fish only contained toxin in their flesh if their livers were also toxic. Furthermore, ciguatoxin concentration was always higher in viscera than in flesh (at least twice as toxic).

The most toxic species (1–10 in Table 1) are large piscivorous species except for *A. afer* which is a small (<300g) invertebrate feeder.

Intermediate toxicity was detected in species 11–15 (Table 1). Lower toxicity was present in other species (16–30 in Table 1). The above classes include small species (<500g) such as *M. martinicus*, *P. arenatus*, *B. rufus* and *H. radiatus* and *M. plumieri* (<1kg) which feed mainly on benthic invertebrates; these species can be more toxic than some larger piscivorous species. Only one species of herbivorous fish, the ocean surgeonfish (29 in Table 1), had low toxicity. No toxicity was found in other herbivorous fish (31–35 in Table 1). The longjaw squirrelfish (36 in Table 1), a specific crustacean feeder, was not toxic.

Statistical analysis of variance of individual toxicity for species 1–11 (Table 1) (independent of weight) indicated that the means were significantly different ($p < 0.01$) for the 11 species group unless *C. latus* and *C. bartholomaei* were removed from this group. Without *C. latus*, the F test was not significant ($p = 0.13$) but the K-W test was significant ($p = 0.04$). This discrepancy can be explained by the difference in size between the *C. bartholomaei* sample ($n = 45$) and the other

TABLE 1. Flesh ciguatoxicity in various fish species from Saint Barthelemy Island: results presented in decreasing order according to toxicity.

No.	Species	Specimen weight	TOXIN CONCENTRATION IN MUg/G OF FLESH							
			<0.05	0.05–0.49	0.5–0.99	1–1.49	1.5–1.99	2–2.99	3–5	>10
1	<i>Seriola dumerili</i>	6–29			1				1	
2	<i>Caranx latus</i>	1.3–6		1	8	8	1	2	4	
3	<i>Caranx ruber</i>	0.75–2.3		4		1		1		
4	<i>Caranx bartholomaei</i>	0.75–4.8		12	14	12	4	2	1	
5	<i>Alectis crinitus</i>	3.3–12		5			1	1		
6	<i>Sphyræna barracuda</i>	3–10		2	2	1				1
7	<i>Epinephelus morio</i>	6.5–8		2	1			1		
8	<i>Alphesites afer</i>	0.1–0.25		(16)(11)(12)(2)	(6)	(2)				
9	<i>Scomberomorus cavalla</i>	15–20			1	3				
10	<i>Scomberomorus regalis</i>	3–4	1	1		1				
11	<i>Gymnothorax funebris</i>	3.5–14.5		6	2					
12	<i>Malacanthus plumieri</i>	0.3–0.6		(5)(20)(5)(4)(6) (4)(2)(12)	(5)(15)					
13	<i>Lutjanus jocu</i>	1.7–2.2		1	2					
14	<i>Lutjanus griseus</i>	1.9–2		2	1					
15	<i>Lutjanus buccanella</i>	0.3–1.5		(3)1(2)	(2)					
16	<i>Priacanthus arenatus</i>	0.4–0.6		(3)(2)						
17	<i>Bodianus rufus</i>	0.2–0.4		(2)(15)(12)						
18	<i>Halichoeres radiatus</i>	0.5–1		(4)(3)						
19	<i>Gymnothorax moringa</i>	1.5–2		2						
20	<i>Mulloidichthys martinicus</i>	0.2–0.4		(20)(20)						
21	<i>Mycteroperca venenosa</i>	3.2–4.5		3						
22	<i>Caranx lugubris</i>	2.5		1						
23	<i>Lutjanus analis</i>	4–5		2						
24	<i>Seriola rivoliana</i>	2.7–4.5		2						
25	<i>Mycteroperca tigris</i>	0.7		(2)						
26	<i>Epinephalus guttatus</i>	0.7–0.9		(3)						
27	<i>Epinephalus adscension</i>	0.5–0.7		(4)						
28	<i>Calamus calamus</i>	0.2–0.4		(6)						
29	<i>Acanthurus bahianus</i>	0.05–0.15		(37)(6)						
30	<i>Balistes vetula</i>	1.5–2	1	1						
31	<i>Acanthurus chirurgus</i>	0.2–0.4	(3)(4)(7)							
32	<i>Acanthurus coeruleus</i>	0.2–0.4	(10)							
33	<i>Scarus coeruleus</i>	2	1							
34	<i>Scarus vetula</i>	0.6	(2)							
35	<i>Sparisoma viride</i>	1	1							
36	<i>Holocentrus ascensionis</i>	0.1–0.2	(11)							

Specimens were tested individually except where indicated by brackets in which case the number of pooled specimens is given.

samples ($n < 10$). *Caranx latus* had the highest mean toxicity of species examined, suggesting that it is the most dangerous species at Saint Barthelemy.

The relationship between individual toxicity and weight was investigated within each of nine species for which $n > 2$; the smooth curves (Fig.3) were fitted with the linear regression model:

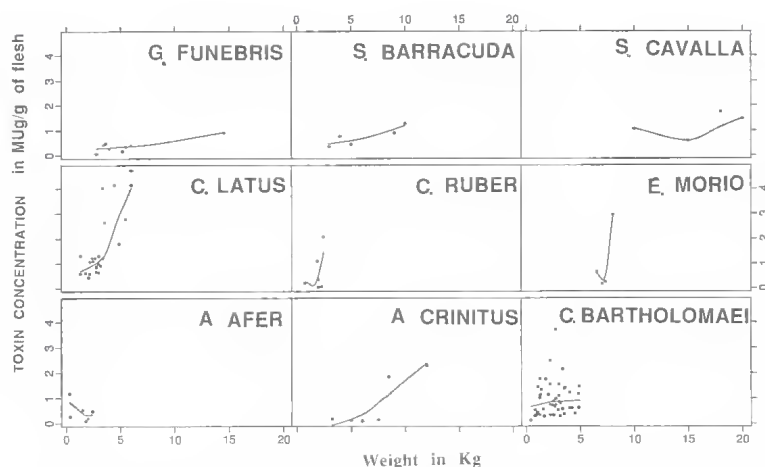


FIG.2. Relation between weight and toxicity for some toxic fish species.

$$\text{toxicity} = \beta_0 + \beta_1 \text{ weight} + E,$$

where toxicity is in MUg/g and weight is in kg. A significant linear correlation between toxicity and weight was found ($\beta_1/0$) only for *C. latus* ($p < 0.01$; $\beta_0 = -0.85502$; $\beta_1 = 0.79673$) and *A. crinitus* ($p = 0.014$; $\beta_0 = -1.31314$; $\beta_1 = 0.29066$).

USE OF BIO-INDICATORS

Since jacks occupy the upper part of the ciguatera food chain, we chose *C. bartholomaei* and *C. latus* adults ($>1\text{kg}$) to investigate the degree of bioaccumulation of ciguatera toxin per

year. From 1979–1985 the median concentration per year of ciguatera toxin in their flesh ($n=62$) was stable around 1 MUg/g of flesh. During the same period other bioindicator species such as *M. plumieri* and *A. afer* (benthic fishes) which were thought to feed directly on the ciguatera toxin producers were also studied. Their toxicity level per year was constantly 0.2–0.6 MUg/g of flesh. So the amount of toxins in the food chain at Saint Barthelemy appeared stable over this period. Nevertheless, in the last years of this study toxicity of *C. latus*

was 3–4 times higher ($n=10$), but no change was found in the toxicity of *C. bartholomaei* ($n=15$).

DISTRIBUTION OF TOXINS AND *G. TOXICUS* IN GUT CONTENTS OF HERBIVOROUS FISHES AND IN CORAL SAMPLES

Extracts of coral samples collected at Saint Barthelemy blanketed with algae did not contain detectable ciguatera toxin, even though *G. toxicus* was present in low numbers on these samples.

The maitotoxin and ciguatera toxin analysis of gut contents of herbivorous fish, obtained in 1975–1976 from French Polynesia (Fig.3) shows that materials ingested by parrotfish *S. gibbus* or surgeonfish *C. striatus* contained ciguatera toxin (fat soluble toxin) and maitotoxin (acetone precipitated toxin). Furthermore, the concentration of ciguatera toxin in gut contents of parrotfish was always higher than that of maitotoxin, whereas the ratio of the two toxins was reversed in the gut contents of the surgeonfish. Thus ciguatera toxin level appears unrelated to maitotoxin level.

CTX content (in MUg) is quantitatively dominant in scraped coral substrate and in the fraction $>85\mu\text{m}$ (=algae+detritus), four times as much as in the *G. toxicus* fraction (Fig.4A). Attempts to remove CTX from coral substrate by scraping in the presence of a low pressure spray of water were unsuccessful: 30–60% of total CTX content remained attached to the coral substrate (3 experiments). With crude material (not frozen) results were

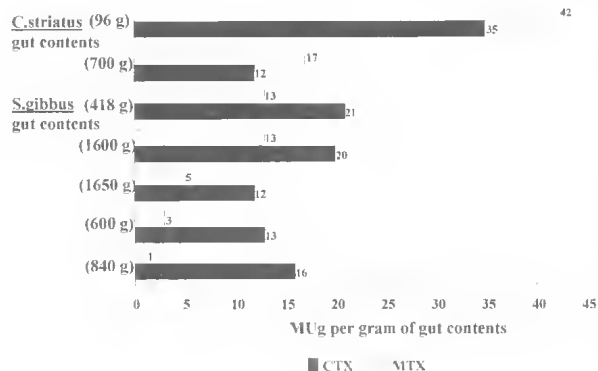


FIG.3. Distribution of toxins in gut contents of surgeonfish *C. striatus* and parrotfish *S. gibbus* caught at the same place in the Gambier Islands.

similar. The MTX content was well correlated with *G. toxicus* (which accumulated in 36–85 μm fraction) and the ratio of MTX to CTX was >1 as it was for the gut toxin contents of *C. striatus*.

Specific CTX content (in MUg/g of dry matter) seems to reside not only with the *G. toxicus* fraction but also on the other size fractions, particularly the fraction $<36\mu\text{m}$ (particles + washing water) (Fig.4b).

G. toxicus thecal structure can be extremely resistant to different conditions: freezing, water dilution, acetone extraction and ultra-turrax sonication.

DISCUSSION

The involvement of fat soluble ciguatoxins in ciguatera in the Caribbean has been confirmed by chemical studies on the toxins in *C. bartholomaei* (Vernoux et al.,1982), *B. rufus*, *M. martinicus*, *M. plumieri*, *E. morio*, *G. funebris*, *S. barracuda*, *S. cavalla*, *S. dumerili* (Vernoux & Abbad,1986), and *A. crinitus*, *C. latus* (Vernoux & Talha,1989). In the Virgin Islands, similar species were clinically found to cause ciguatera poisoning (Brody, 1971; Morris et al.,1982; Engleberg et al.,1983) and ciguatoxin from *Lutjanus buccanella* has been well documented (Hoffman et al.,1983).

At Saint Barthelemy the same species of fish were ciguatoxic as in the Pacific (Randall,1958; Halstead,1978). Nevertheless, jacks are much more toxic in the Caribbean (Arcisz,1950) than in the Pacific Ocean (*Caranx ignobilis* was the only species suspected in the Pacific by Bagnis (1981)). The importance of feeders on small benthic invertebrate feeders in the ciguatera food chain at Saint Barthelemy is worth pointing out since this suggests that ciguatera transmission begins primarily at the invertebrate level. Invertebrates appear to be less important in the Pacific area, though some feeders on invertebrates, such as *Lethrinus kallopterus* at the Marshall Islands (Randall,1980), *Cheilinus undulatus* at Tahiti (Bagnis,1968) and other Lethrinidae at New Caledonia (Bagnis,1979a) are ciguatoxic.

In the French West Indies all ciguateric species were shore fish associated with reefs. With the exception of two semi-pelagic open water species, the king mackerel and cero, they were bottom dwelling species generally found at a depth of $<50\text{m}$ ($>100\text{m}$ for black jack and greater amberjack). Ciguatoxins are therefore well correlated with the benthic fish. Further illustration is provided by *Caranx ruber* which is dangerous at Saint Barthelemy when caught in fish traps

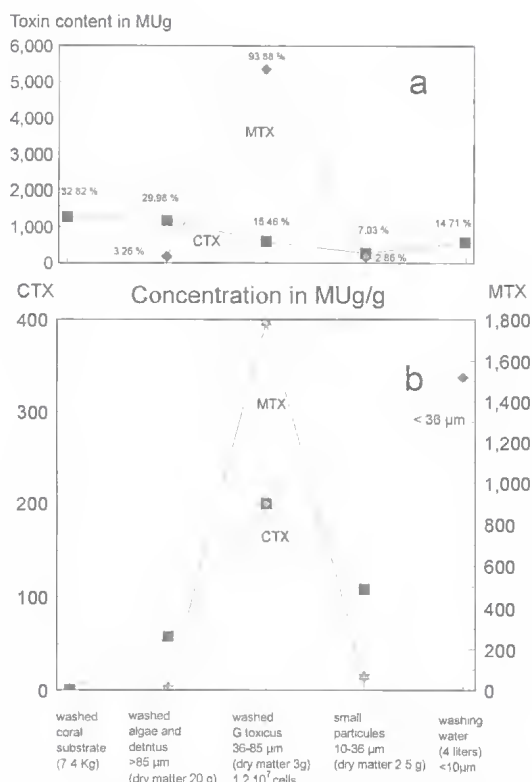


FIG. 4. Distribution of toxins MTX and CTX in fractionated material from the Gambier Islands either considering total toxin content (a) or toxin concentration (b).

(sedentary individuals) but not in nets (migrating shoals of fish).

Unlike the Pacific, at Saint Barthelemy herbivorous fish are regularly consumed without suspicion. Similar observations have been reported in other areas of the Caribbean (Bagnis 1979a; Czernichow et al.,1984) in New Caledonia (Bagnis,1979b) and in the Indian Ocean (Lebeau & Telmar,1978). Nevertheless detectable ciguatoxicity in the surgeonfish *A. bahianus* indicates that low (subsymptomatic) ciguatoxin levels may be present in some Caribbean herbivorous fish species, illustrating that fish edibility depends on toxin level as already described (Bagnis & Vernoux,1975). Here we cannot exclude the possibility that ciguatoxin is present in the other herbivorous fishes, though ciguatoxin levels are probably (extremely) low. The higher toxicity of *A. bahianus* suggests a different diet. We studied 3 Caribbean surgeonfishes (Stokes,1980; Randall,1983) that have different feeding habits (Randall,1967): *A.*

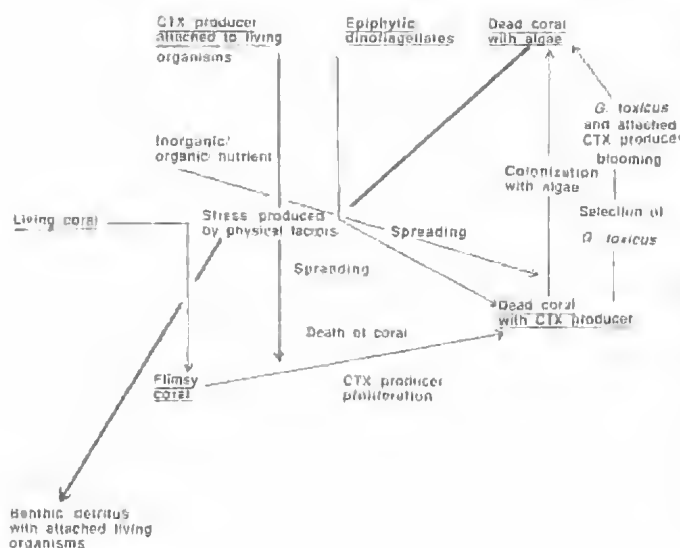


FIG. 5. A proposed mechanism for the initiation of CTX producer proliferation.

chirurgus and *A. bahianus* has a thick walled gizzard-like stomach and ingests inorganic sediment (sand, small shells etc.) with the algae they crop from solid substrata (especially *A. bahianus*) (in Randall, 1967; and personal observations); and *A. coeruleus* has a thin walled stomach in which we have observed a higher content of green algae and it does not usually ingest sand. The former are therefore grazers whereas the latter is a browser following the definition of Lobel (1981). In the West Indies, parrotfishes are also grazers that feed mainly on algae attached to dead coral (Randall, 1967), except for large *Sparisoma viride* and occasionally large *Scarus vetula* which graze on live coral (Frydl, 1979). Taking into account these feeding habits and the corresponding toxicity results, we deduce that the source of the toxin in the food could be an organism linked to bottom detritus. The ciguatoxin producer *G. toxicus*, found on dead coral (Bagnis et al., 1980) and closely associated with reef sediments or with macroalgae (Taylor, 1979; Yasumoto et al., 1979) may be the ciguatoxin-producer at Saint barthelemy. This conclusion is consistent with the presence of *G. toxicus* (Bagnis, 1981; Besada et al., 1982; Bourdeau & Durand-Clement, 1991) and with previous studies (Yasumoto et al., 1977b; Bagnis et al., 1980) which have implicated *G. toxicus* as the ciguatera produced in the Pacific. Nevertheless, the somewhat conflicting results obtained in the study of scraped coral and discussion concerning ingested toxins in gut con-

tents of herbivorous fish at Tahiti have not previously been considered.

Chanteau (1978) studied scraped coral as well as other fractions for toxicity. She obtained toxin partitioning results similar to ours, even though scraping was practised with a metallic brush, i.e. MTX content was highly correlated with the *G. toxicus* containing fraction and <10% of total MTX was present in scraped coral substrate, and in this substrate the proportion of MTX to CTX were inverted (<1). These observations and ours demonstrated that MTX is a *G. toxicus* marker, especially since it is never excreted out of the dinoflagellate (Yasumoto et al., 1979b). However, CTX contamination level was independent of the amount of *G. toxicus* in these studies. This latter statement was corroborated by analysis of toxins in the gut contents of herbivorous fish: our results and

those of others (Yasumoto et al., 1975; Yasumoto et al. 1977a) showed that the relative proportions of MTX and CTX are inverted in surgeonfish (*C. striatus*) compared with parrotfish (*S. gibbus*) caught in the same area. The difference in proportion corresponds to the difference in their feeding habits: the former is a browser which ingests chlorophyll-bearing material (10%) as well as detritus and numerous *G. toxicus* were found in its stomach and gut contents, while the parrotfish is a coral feeder exclusively (103–260 µg of algae/100g of ingested sample according to Yasumoto et al. (1977a) and no *G. toxicus* was visible in its stomach or gut contents (Vernoux, 1981). Thus ciguatoxin producer could be associated also with living coral (perhaps the zooxanthellae?). Bagnis et al. (1980) stated that they could not find any indication that CTX (or MTX) was excreted into the culture medium, while Shimizu et al. (1982) and Campbell et al. (1987) using fluorescence labelled sheep anti-ciguatoxin antibody both found that the outer wall of a certain percentage of *G. toxicus* contained ciguatoxin and/or ciguatoxin-like compounds. Thus the ciguatoxin producer could be a very small organism dependent (or not) on certain epiphytic dinoflagellates such as *G. toxicus* and perhaps zooxanthellae. The findings that (i) ciguatoxin inhibits cellular multiplication of unicellular marine algae (Durand et al., 1985), (ii) coral death often seems necessary to induce

ciguatera production (Bagnis, 1981). (iii) dead coral provides new surfaces for dinoflagellates implicated in ciguatera fish poisoning (Kohler & Kohler, 1992) and (iv) the presence of CTX in dead corals with *G. toxicus* together suggest that the ciguatoxin producer could contribute to the death of living coral which could in turn enhance *G. toxicus* proliferation (Fig. 5).

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